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Complete genome sequence of *Spirochaeta smaragdinae* type strain (SEBR 4228^T)

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Spirochaeta smaragdinae Magot *et al.* 1998 belongs to the family *Spirochaetaceae*. The species is Gram-negative, motile, obligately halophilic and strictly anaerobic and is of interest because it is able to ferment numerous polysaccharides. *S. smaragdinae* is the only species of the family *Spirochaetaceae* known to reduce thiosulfate or element sulfur to sulfide. This is the first complete genome sequence in the family *Spirochaetaceae*. The 4,653,970 bp long genome with its 4,363 protein-coding and 57 RNA genes is a part of the *Genomic Encyclopedia of Bacteria and Archaea* project.

Introduction

Strain SEBR 4228^T (= DSM 11293 = JCM 15392) is the type strain of the species *Spirochaeta smaragdinae*. Currently, there are eighteen species [1] and two subspecies in the genus *Spirochaeta* [1,2]. The generic name derives from the Greek word ‘*speira*’ meaning ‘a coil’ and the Greek word ‘*chaitê*’ meaning ‘hair’, referring to the spiral shape of bacterial cell. The species epithet is derived from the Latin word ‘*smaragdinae*’ meaning ‘from Emerald’, referring to the name Emerald of an oil field in Congo. Strain SEBR 4228^T was isolated from an oil-injection production water sample of a Congo offshore oilfield [3] and described in 1997 by Magot *et al.* as ‘*Spirochaeta smaragdi-*

nae’ [3]. Here we present a summary classification and a set of features for *S. smaragdinae* SEBR 4228^T, together with the description of the complete genomic sequencing and annotation.

Classification and features

Strain SEBR 4228^T shares 82.2–99.0% 16S rRNA gene sequence identity with the type strains from the other members of genus *Spirochaeta* [4], with the type strain of *S. bajacaliforniensis* [5], isolated from a mud sample in Laguna Figueroa (Baja California, Mexico) showing the highest degree of sequence similarity (99%). Notwithstanding the high degree of 16S rRNA gene sequence identity,

these two strains are characterized by low genomic similarity (38%) in DNA-DNA hybridization studies and differ by numerous differences in carbon source utilization [3]. Several type strains from the genus *Treponema* show the highest degree of similarity for non-*Spirochaeta* strains (82.9-83.6%) [4]. A representative genomic 16S rRNA sequence of strain SEBR 4228^T was compared using BLAST with the most recent release of the Greengenes database [6] and the relative frequencies of taxa and keywords, weighted by BLAST scores, were determined. The three most frequent genera were *Spirochaeta* (76.4%), '*Sphaerochaeta*' (15.8%) and *Cytophaga* (7.8%). Within the five most frequent keywords in the labels of environmental samples were 'microbial' (11.7%), 'mat' (10.5%), 'hypersaline' (7.7%), and

'sediment' (1.7%). The environmental samples database (env_nt) contains the marine metagenome genomic clone 1061006082084 (EK988302) that is 92% identical to the 16S rRNA gene sequence of SEBR 4228^T. No phylotypes from genomic surveys could be linked to the species *S. smaragdinae* or even the genus *Spirochaeta*, indicating a rather rare occurrence of these in the habitats screened so far (as of August 2010).

Figure 1 shows the phylogenetic neighborhood of *S. smaragdinae* SEBR 4228^T in a 16S rRNA based tree. The sequences of the two 16S rRNA gene copies differ from each other by up to one nucleotide, and differ by up to five nucleotides from the previously published 16S rRNA sequence generated from DSM 11293 (U80597), which contains two ambiguous base calls.

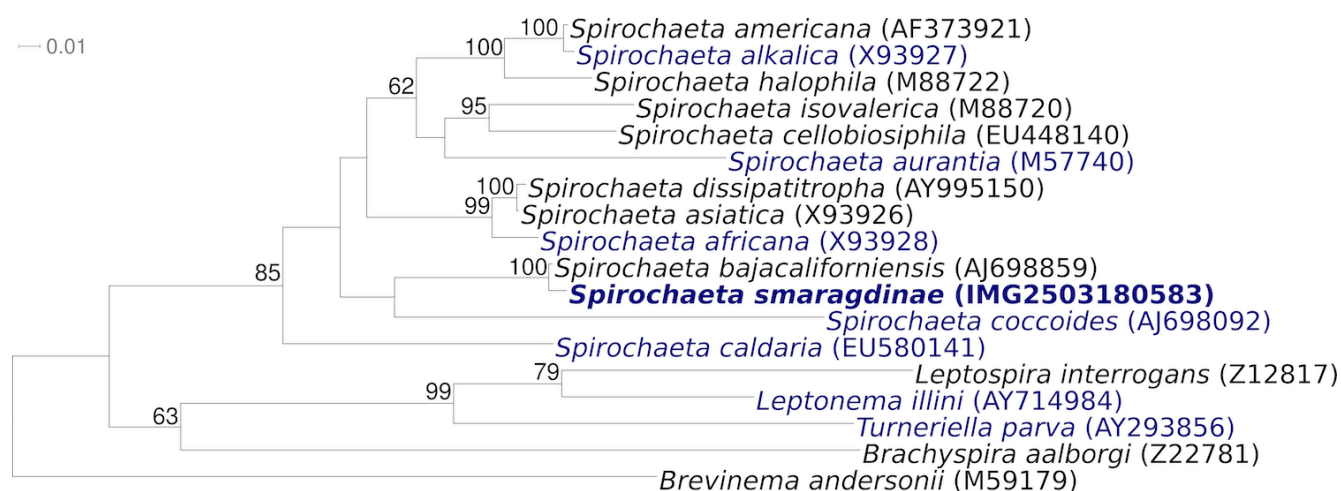


Figure 1. Phylogenetic tree highlighting the position of *S. smaragdinae* SEBR 4228^T relative to the type strains of the other species within the genus and of the other genera within the genus *Spirochaeta*. The tree was inferred from 1,385 aligned characters [7,8] of the 16S rRNA gene sequence under the maximum likelihood criterion [9] and rooted in accordance with the current taxonomy [10]. The branches are scaled in terms of the expected number of substitutions per site. Numbers above branches are support values from 500 bootstrap replicates [11] if larger than 60%. Lineages with type strain genome sequencing projects registered in GOLD [12] are shown in blue, published genomes in bold.

Strain SEBR 4228^T is a Gram-negative, chemotrophic and strictly anaerobic bacterium with spiral shaped, 0.3-0.5 × 5-30 µm long cells (Figure 2 and Table 1). It possesses a multilayer, crenulating, Gram-negative cell envelope, which consists of an outer membrane and an inner membrane adjoining the cytoplasmic membrane [3]. Sillons, which are the contact point between the protoplasmic cylinder, the inner membrane and the outer membrane, are also observed from the cells of *S. smaragdinae* SEBR 4228^T [3]. Strain SEBR 4228^T forms translucent colonies with regular edges (0.5

mm of diameter) after two weeks of incubation on SEM agar plates at 37°C [3]. The strain is motile with a corkscrew-like motion, which is characteristic for the typical 1-2-1 periplasmic flagellar arrangement of the members of the genus *Spirochaeta* [3]. The periplasmic, non-extracellular location of the flagella make the *Spirochaeta* a valuable candidate for the study of flagella evolution [26]. The enlarged spherical bodies, which are typical for spirochetes, are also observed in strain SEBR 4228^T [3]. The temperature range for growth is from 20°C to 40°C, with an optimum

temperature at 37°C [3]. The pH range for growth is between 5.5 and 8.0, with an optimum pH of 7.0 [3]. Strain SEBR 4228^T is obligately halophilic [3] and is able to grow on media that contains 1-10% of NaCl, with an optimum salinity at 5% NaCl [3]. Under optimum growth conditions, the doubling time is approximately 25 h in the presence of glucose and thiosulfate [3]. Strain SEBR 4228^T is able to utilize biotrypcase, fructose, fumarate, galactose, D-glucose, glycerol, mannitol, mannose, ribose, D-xylose and yeast extract, but not acetate, D-arabinose, butyrate, casamino acids, lactate, maltose, propionate, pyruvate, rhamnose, sorbose, sucrose and L-xylose [3]. Yeast extract is required for growth and cannot be replaced by a vitamin

mixture [3]. Strain SEBR 4228^T ferments fumarate to acetate and succinate [3]. The major end-product of glucose fermentation of strain SEBR 4228^T is lactate with traces of H₂ and ethanol [3]. *S. smaragdinae* is the only species of *Spirochaeta* known to reduce thiosulfate or elemental sulfur to sulfide [3]. Strain SEBR 4228^T produces lactate, acetate, CO₂ and H₂S as the end-products of glucose oxidation when thiosulfate is present in the growth medium [3]. The strain contains a rhodanese-like protein which expresses rhodanese activity [27]. This enzyme is able to reduce thiosulfate to sulfide [28]. Rhodanese is also widely found in other members of the domain *Bacteria* [29-31].

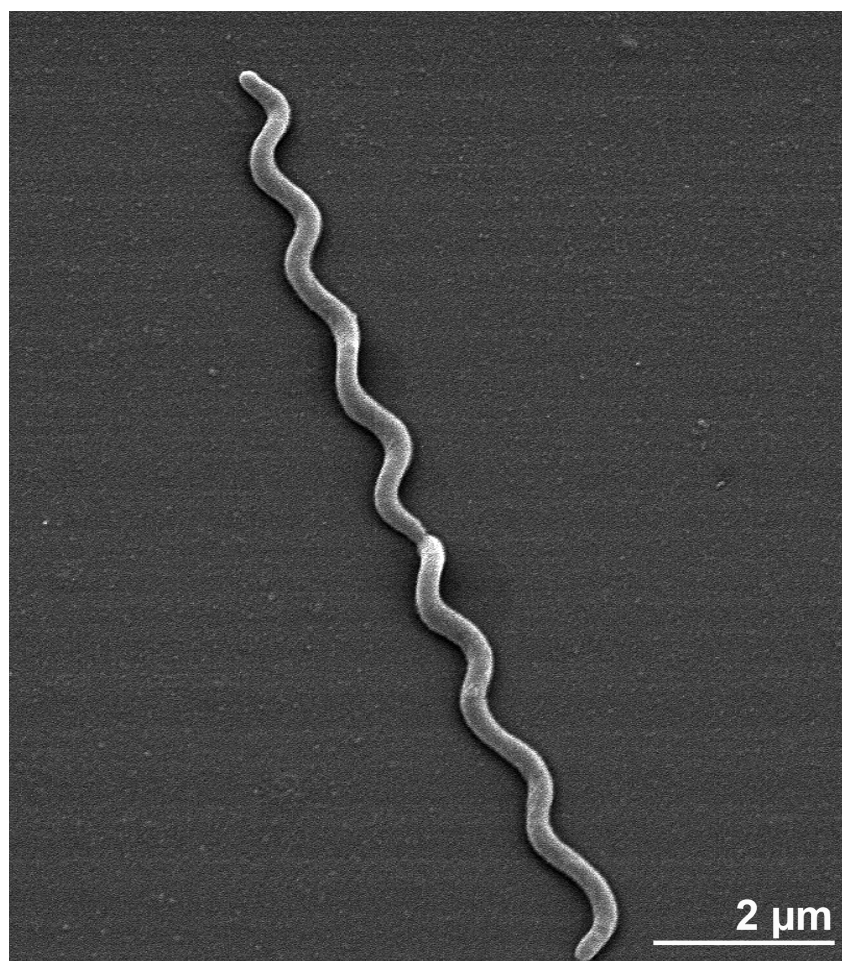


Figure 2. Scanning electron micrograph of *S. smaragdinae* SEBR 4228^T

Chemotaxonomy

No cellular fatty acids profiles are currently available for *S. smaragdinae* SEBR 4228^T. However, C_{16:0} dimethyl acetate is the major cellular fatty acids of the type strains of the closely related *S.*

dissipatitropa, *S. asiatica* and *S. americana*, and C_{16:0} fatty acid methyl ester is the major cellular fatty acids of *S. africana* [20,32].

Table 1. Classification and general features of *S. smaragdinae* SEBR 4228^T according to the MGS recommendations [13].

| MIGS ID | Property | Term | Evidence code |
|----------|------------------------|---|----------------|
| | | Domain <i>Bacteria</i> | TAS [14] |
| | | Phylum <i>Spirochaetae</i> | TAS [15,16] |
| | | Class <i>Spirochaetes</i> | TAS [16] |
| | Current classification | Order <i>Spirochaetales</i> | TAS [17,18] |
| | | Family <i>Spirochaetaceae</i> | TAS [18,19] |
| | | Genus <i>Spirochaeta</i> | TAS [18,20-22] |
| | | Species <i>Spirochaeta smaragdinae</i> | TAS [3,23] |
| | | Type strain SEBR 4228 | TAS [3] |
| | Gram stain | negative | TAS [3] |
| | Cell shape | spiral | TAS [3] |
| | Motility | yes | TAS [3] |
| | Sporulation | none | NAS |
| | Temperature range | between 20°C and over 40°C | TAS [3] |
| | Optimum temperature | 37°C | TAS [3] |
| | Salinity | 1-10% NaCl (optimum 5%) | TAS [3] |
| MIGS-22 | Oxygen requirement | obligately anaerobic | TAS [3] |
| | Carbon source | polysaccharides | TAS [3] |
| | Energy source | chemoorganotroph | TAS [3] |
| MIGS-6 | Habitat | oil-fields | TAS [3] |
| MIGS-15 | Biotic relationship | free-living | TAS [3] |
| MIGS-14 | Pathogenicity | none | NAS |
| | Biosafety level | 1 | TAS [24] |
| | Isolation | oil-injection water sample in the production system of an oil field | TAS [3] |
| MIGS-4 | Geographic location | Emerald oil fields in Congo | TAS [3] |
| MIGS-5 | Sample collection time | 1997 or before | TAS [3] |
| MIGS-4.1 | Latitude | not reported | |
| MIGS-4.2 | Longitude | not reported | |
| MIGS-4.3 | Depth | not reported | |
| MIGS-4.4 | Altitude | not reported | |

Evidence codes - IDA: Inferred from Direct Assay (first time in publication); TAS: Traceable Author Statement (i.e., a direct report exists in the literature); NAS: Non-traceable Author Statement (i.e., not directly observed for the living, isolated sample, but based on a generally accepted property for the species, or anecdotal evidence). These evidence codes are from of the Gene Ontology project [25]. If the evidence code is IDA, then the property was directly observed by one of the authors or an expert mentioned in the acknowledgements.

Genome sequencing and annotation

Genome project history

This organism was selected for sequencing on the basis of its phylogenetic position [33], and is part of the *Genomic Encyclopedia of Bacteria and Archaea* project [34]. The genome project is deposited in the Genome OnLine Database [12] and the

complete genome sequence is deposited in GenBank. Sequencing, finishing and annotation were performed by the DOE Joint Genome Institute (JGI). A summary of the project information is shown in Table 2.

Table 2. Genome sequencing project information

| MIGS ID | Property | Term |
|-----------|----------------------------|---|
| MIGS-31 | Finishing quality | Finished |
| MIGS-28 | Libraries used | Three genomic libraries: 454 pyrosequence standard and PE (12 kb insert size) libraries and one Illumina standard library |
| MIGS-29 | Sequencing platforms | 454 GS FLX Titanium, Illumina GAii |
| MIGS-31.2 | Sequencing coverage | 58.8 × pyrosequence, 6.9 × Illumina |
| MIGS-30 | Assemblers | Newbler version 2.0.0-PostRelease-11/04/2008, phrap, |
| MIGS-32 | Gene calling method | Prodigal 1.4, GenePRIMP |
| | INSDC ID | CP002116 |
| | Genbank Date of Release | August 6, 2010 |
| | GOLD ID | Gc013354 |
| | NCBI project ID | 32637 |
| | Database: IMG-GEBA | 2503128010 |
| MIGS-13 | Source material identifier | DSM 11293 |
| | Project relevance | Tree of Life, GEBA |

Growth conditions and DNA isolation

S. smaragdinae SEBR 4228^T, DSM 11293, was grown anaerobically in medium 819 (*Spirochaeta smaragdinae* medium) [35] at 35°C. DNA was isolated from 0.5-1 g of cell paste using MasterPure Gram Positive DNA Purification Kit (Epicentre MGP04100) following the standard protocol as recommended by the manufacturer, with modification st/LALMice for cell lysis as described in Wu *et al.* [34].

Genome sequencing and assembly

The genome was sequenced using a combination of Illumina and 454 sequencing platforms. All general aspects of library construction and sequencing can be found at the [JGI website](#). Pyrosequencing reads were assembled using the Newbler assembler version 2.0.0-PostRelease-11/04/2008 (Roche). The initial Newbler assembly consisted of 51 contigs in one scaffold was converted into a phrap assembly by making fake reads from the consensus, collecting the read pairs in the 454 paired end library. Illumina GAii sequencing data was assembled with Velvet [36] and the consensus sequences were shredded into 1.5 kb overlapped fake reads and assembled together with the 454 data. Draft assemblies were based on 273 Mb 454 draft data and all of the 454 paired end data. Newbler parameters are -consed -a 50 -l 350 -g -m -ml 20.

The [Phred/Phrap/Consed](#) software package was used for sequence assembly and quality assessment in the following finishing process. After the shotgun stage, reads were assembled with parallel phrap (High Performance Software, LLC). Possible mis-assemblies were corrected with [gapResolution](#), Dupfinisher, or sequencing cloned bridging PCR fragments with subcloning or transposon bombing (Epicentre Biotechnologies, Madison, WI) [37].

Gaps between contigs were closed by editing in Consed, by PCR and by Bubble PCR primer walks (J.-F.Chang, unpublished). A total of 147 additional reactions were necessary to close gaps and to raise the quality of the finished sequence. Illumina reads were also used to improve the final consensus quality using an in-house developed tool - the Polisher [38]. The error rate of the completed genome sequence is 0.2 in 100,000. Together, the combination of the Illumina and 454 sequencing platforms provided 65.7× coverage of the genome.

Genome annotation

Genes were identified using [Prodigal](#) [39] as part of the Oak Ridge National Laboratory genome annotation pipeline, followed by a round of manual curation using the JGI [GenePRIMP](#) pipeline [40]. The predicted CDSs were translated and used to search the National Center for Biotechnology Information (NCBI) nonredundant database, UniProt, TIGRFam, Pfam, PRIAM, KEGG, COG, and InterPro databases. Additional gene prediction analysis and functional annotation was performed within the Integrated Microbial Genomes - Expert Review (IMG-ER) platform [41].

Genome properties

The genome consists of a 4,653,970 bp long chromosome with a 49.0% GC content (Table 3 and Figure 3). Of the 4,363 genes predicted, 4,306 were protein-coding genes, and 57 RNAs; eighty seven pseudogenes were also identified. The majority of the protein-coding genes (74.2%) were assigned with a putative function while the remaining ones were annotated as hypothetical proteins. The distribution of genes into COGs functional categories is presented in Table 4.

Table 3. Genome Statistics

| Attribute | Value | % of Total |
|----------------------------------|-----------|------------|
| Genome size (bp) | 4,653,970 | 100.00% |
| DNA coding region (bp) | 4,315,215 | 92.97% |
| DNA G+C content (bp) | 2,278,823 | 48.97% |
| Number of replicons | 1 | |
| Extrachromosomal elements | 0 | |
| Total genes | 4,363 | 100.00% |
| RNA genes | 57 | 1.31% |
| rRNA operons | 2 | |
| Protein-coding genes | 4306 | 98.69% |
| Pseudo genes | 87 | 1.99% |
| Genes with function prediction | 3,235 | 74.15% |
| Genes in paralog clusters | 818 | 18.75% |
| Genes assigned to COGs | 3,318 | 76.05% |
| Genes assigned Pfam domains | 3,443 | 78.91% |
| Genes with signal peptides | 871 | 26.36% |
| Genes with transmembrane helices | 1,150 | 22.45% |
| CRISPR repeats | 1 | |

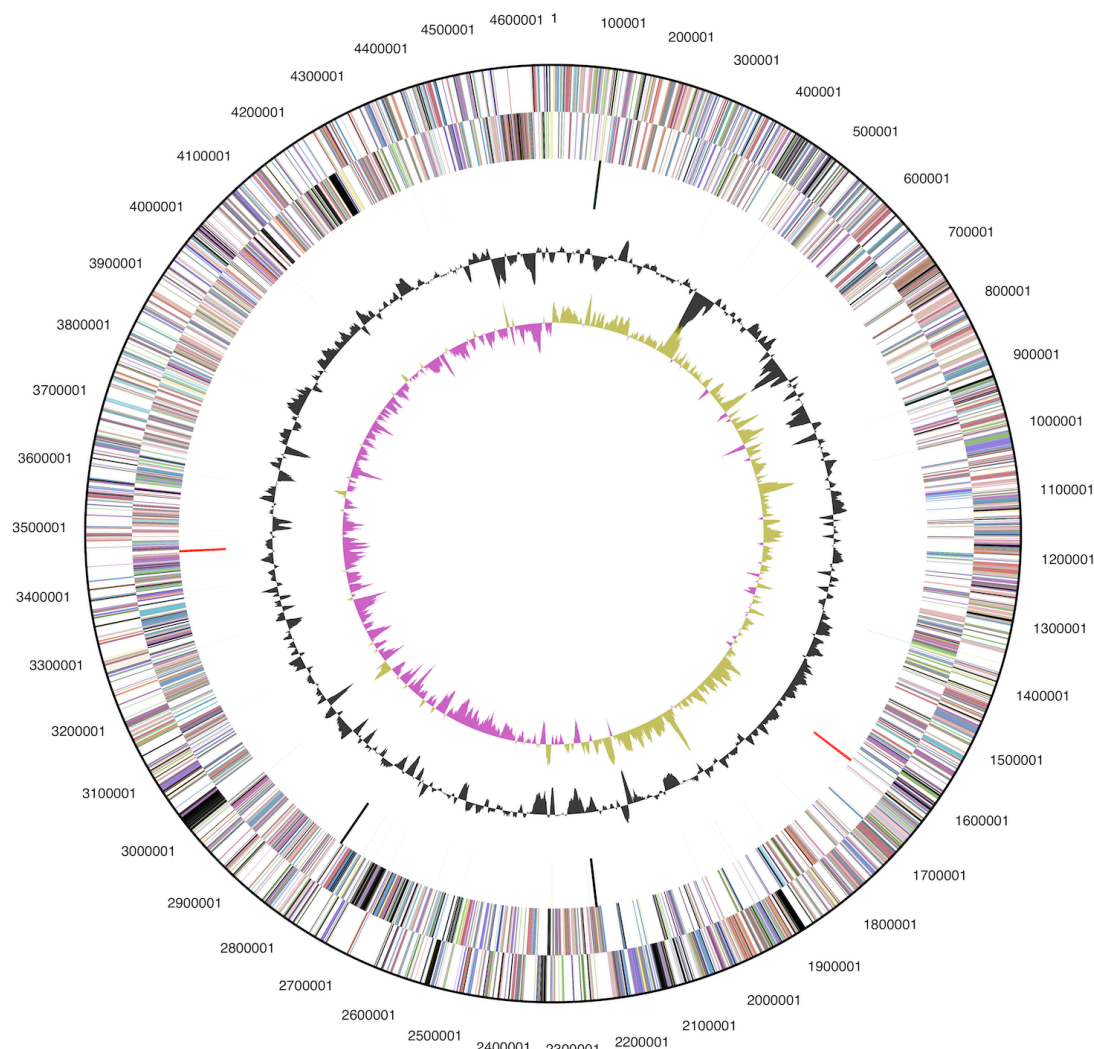


Figure 3. Graphical circular map of the genome. From outside to the center: Genes on forward strand (color by COG categories), Genes on reverse strand (color by COG categories), RNA genes (tRNAs green, rRNAs red, other RNAs black), GC content, GC skew.

Table 4. Number of genes associated with the general COG functional categories

| Code | Value | %age | Description |
|------|-------|------|--|
| J | 159 | 4.3 | Translation, ribosomal structure and biogenesis |
| A | 0 | 0.0 | RNA processing and modification |
| K | 328 | 8.8 | Transcription |
| L | 129 | 3.5 | Replication, recombination and repair |
| B | 1 | 0.0 | Chromatin structure and dynamics |
| D | 25 | 0.7 | Cell cycle control, cell division, chromosome partitioning |
| Y | 0 | 0.0 | Nuclear structure |
| V | 58 | 1.6 | Defense mechanisms |
| T | 321 | 8.6 | Signal transduction mechanisms |
| M | 183 | 4.9 | Cell wall/membrane/envelope biogenesis |
| N | 94 | 2.5 | Cell motility |
| Z | 0 | 0.0 | Cytoskeleton |
| W | 0 | 0.0 | Extracellular structures |
| U | 58 | 1.6 | Intracellular trafficking and secretion, and vesicular transport |
| O | 114 | 3.1 | Posttranslational modification, protein turnover, chaperones |
| C | 223 | 6.0 | Energy production and conversion |
| G | 553 | 14.9 | Carbohydrate transport and metabolism |
| E | 326 | 8.8 | Amino acid transport and metabolism |
| F | 96 | 2.6 | Nucleotide transport and metabolism |
| H | 130 | 3.5 | Coenzyme transport and metabolism |
| I | 61 | 1.6 | Lipid transport and metabolism |
| P | 165 | 4.4 | Inorganic ion transport and metabolism |
| Q | 30 | 0.8 | Secondary metabolites biosynthesis, transport and catabolism |
| R | 450 | 12.1 | General function prediction only |
| S | 212 | 5.7 | Function unknown |
| - | 1,045 | 23.9 | Not in COGs |

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